

Isomer Barrier (Hex)₆

“The number of all possible linear and branched isomers of a hexasaccharide were calculated and found to be $>1.05 \times 10^{12}$.”

“This large number defines the *Isomer Barrier*, a persistent technological barrier to the development of a single analytical method for the absolute characterization of carbohydrates, regardless of sample quantity.”

“No current method of chemical or physical analysis has the resolution necessary to distinguish among 10^{12} structures having the same mass.”

R. Laine, *Glycobiology* 1994

Isomers by energy-resolved mass spectra (ERMS)

- “ERMS has advantages over conventional CID-MSⁿ, because the data deal with spectra over wide range of collision energy.” e.g., MSⁿ lacks differential collisional energies.

$$k_i(E) = \frac{v}{(1 - E_0/E)S}$$

Osamu Kanie, 2006

Isomers and Neutral Gas Collision

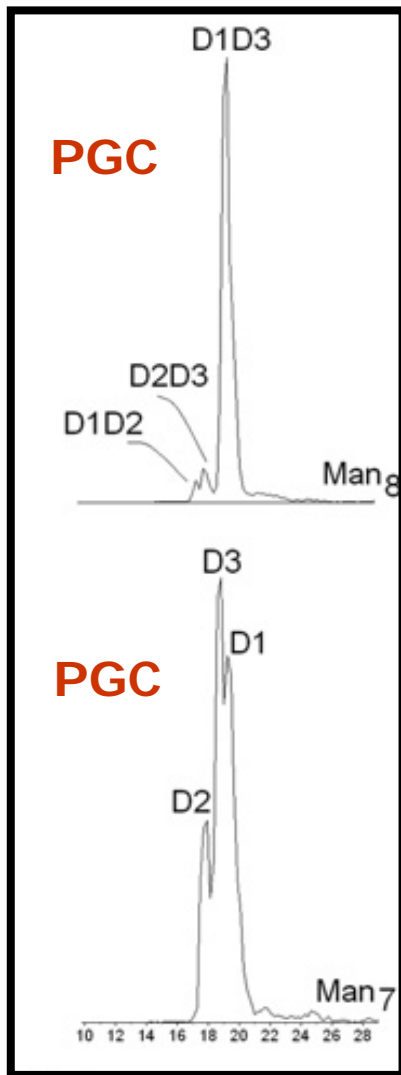
- “...the stereochemical blindness of mass spectrometry, product ions must first be separated based upon a physical principle that is not dependent upon m/z prior to fragmentation.”
- Such skepticism was based on “..a serious problem because they (isomers) yield sets of substructures after every round of dissociation where subsequent fragmentation of any given isolated ion m/z furnishes identical product ion m/z values”

Herb Hill and Brad Bendiak, 2007

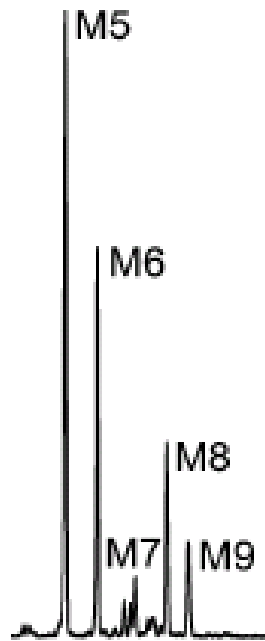
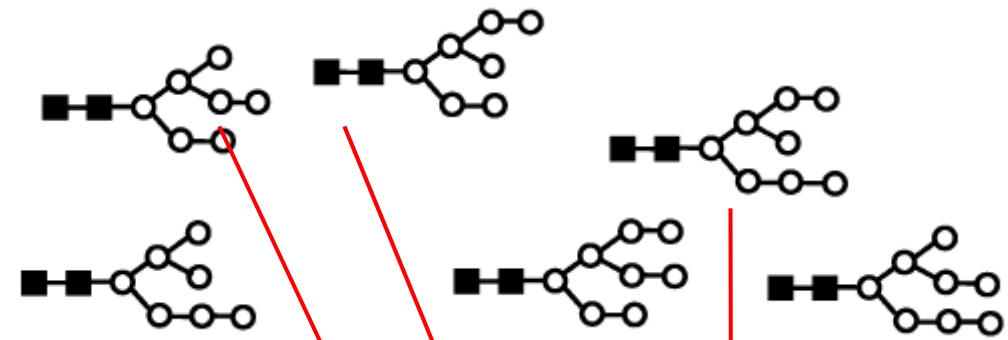
Ribonuclease B Glycosylation

- Fu, D., Chen, L.; O'Neill, R.A. A detailed structural characterization of ribonuclease B oligosaccharides by ¹H NMR spectroscopy and mass spectrometry. *Carbohydr. Res.* **1994**, 261, 173-186.
- Costello, C.E.; Contado-Miller, J.M.; Cipollo, J.F. A glycomics platform for the analysis of permethylated oligosaccharide alditols. *J. Am. Soc. Mass Spectrom.* **2007**, 18, 1799-1812.
- Zhao, C.; Xie, B.; Chan, S.Y.; Costello, C.E.; O'Connor, P.B. Collisionally activated dissociation and electron capture dissociation provide complementary structural information for branched permethylated oligosaccharides. *J. Am. Soc. Mass Spectrom.* **2008**, 19, 138-150.
- Zhuang, Z.; Starkey, J.A.; Mechref, Y.; Novotny, M.V. and Jacobson, S.C. Electrophoretic analysis of N-glycans on microfluidic devices. *Anal. Chem.* **2007**, 79, 7170-7175.

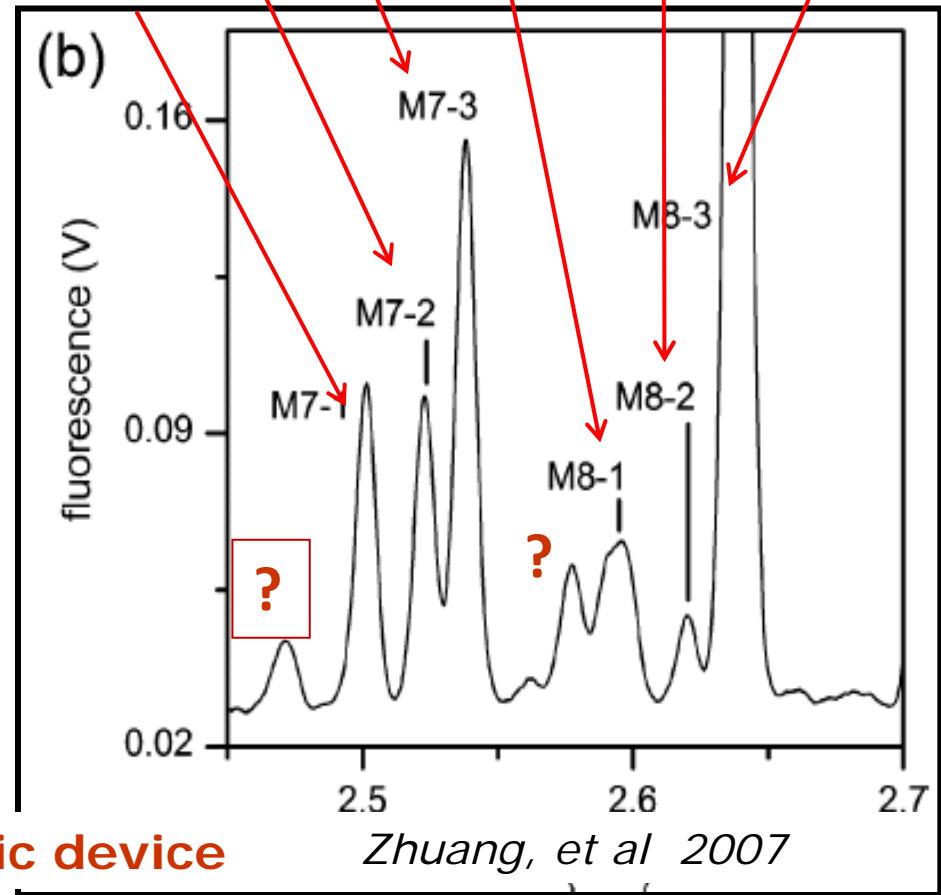
RNase B Glycans



Costello, et al, 2007

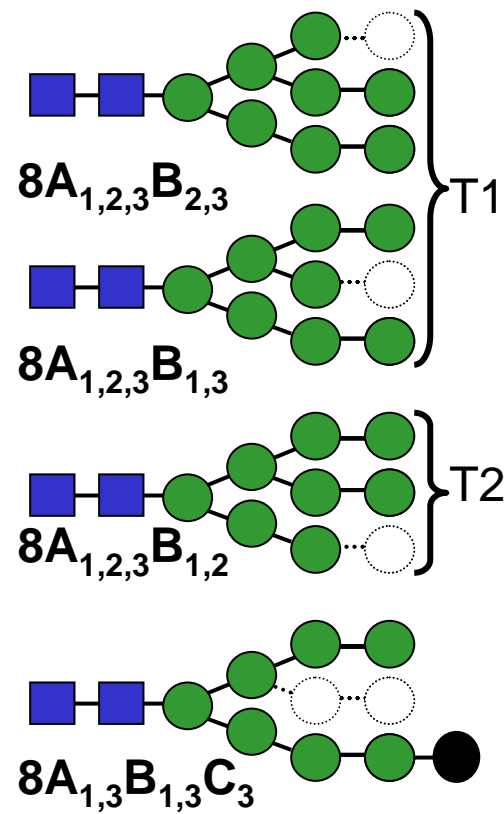
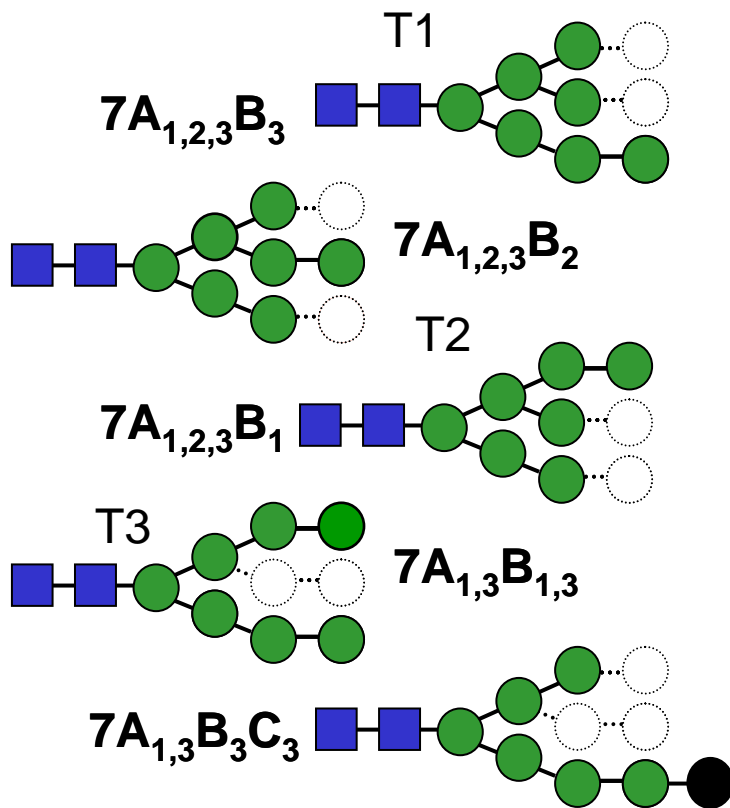
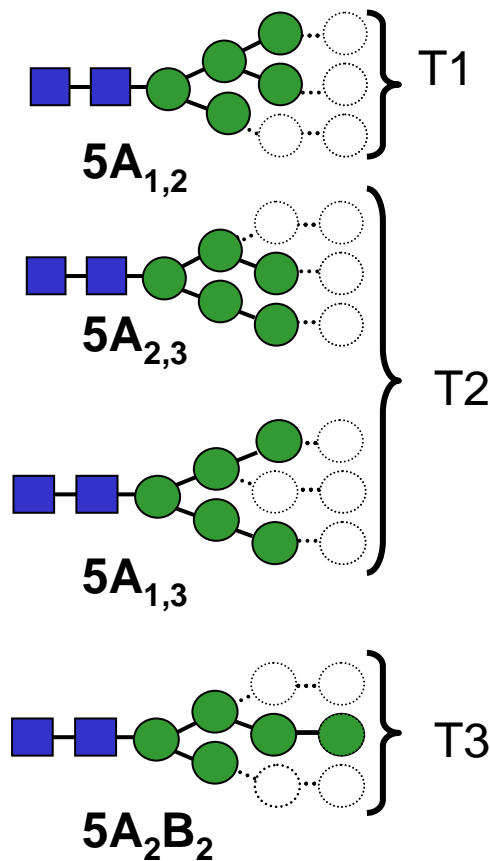
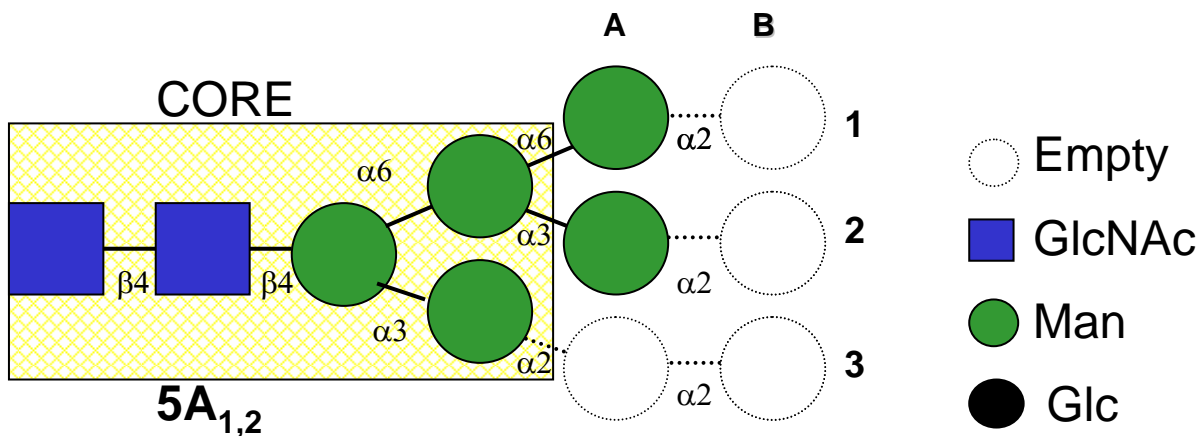


Microfluidic device



Zhuang, et al 2007

Numbering System For High Mannose Isomers



Principles built around ITMS disassembly

- (i.) Collision energies are effectively enhanced with disassembly, providing greater structural detail upon progression to fewer oligomers, (oscillators).
- (ii.) Selected pathways of disassembly can define domains of structure providing antennal specificity
- (iii.) Metal ions (Na^+), enhance sensitivity, fragmentation, and adduct stereo-specifically providing an opportunity to evaluate monomer stereochemistry.
- (iv.) Ion pathways that fail to define a single topology are an indicator of structural isomers, which can, in turn, be isolated and characterized
- (v.) A pathway of disassembly preferentially releases labile residues that dominate spectra providing new products at each step with more comparable stability and equivalent ionization cross-sections, e.g., new sites for metal ion adduction, new fragments.
- (vi.) Samples prepared by methylation and reduction position fragments in a disassembly pathway, thus, termini, extending, and branched components of structure can be distinctly placed.
- (vii) A searchable library of fragments anchors the details of disassembly.